REPLACEMENT OF FISH MEAL WITH PROCESSED POULTRY OFFAL MEAL AND SOYBEAN MEAL IN THE DIETS OF SNAKEHEAD, *Channa striata* (Bloch, 1793) JUVENILE

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by

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ABBREVIATION

AABA	Alpha amino butyric acid
ADM	Apparent dry matter digestibility
ANOVA	One-way analysis of variance
AOAC	Association of official analytical chemists
APD	Apparent protein digestibility
BSA	Bovine serum albumin
CMC	Carboxy methyl cellulose
$CuSO_4$	Copper sulphate
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
EAA	Essential amino acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FAO	Food and agriculture organization
FM	Fish meal
FCR	Food conversion ratio
GC	Gas chromatography
GE	Gross energy
H_2SO_4	Sulphuric acid
Hb	Haemoglobin
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography

HSI	Hepatosomatic index
IPF	Intraperitoneal fat
K_2SO_4	Potasium sulphate
LA	Linoleic acid
LNA	Linolenic acid
LP-POM	Lab processed poultry offal meal
MUFA	Monounsaturated fatty acid
NaOH	Sodium hydroxide
NEAA	Non-essential amino acid
NFE	Nitrogen free extract
PER	Protein efficiency ratio
PEG	Polyethylene glycol
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
SBM	Soybean meal
SD	Standard deviation
SFA	Saturated fatty acid
SGR	Specific growth rate
SPSS	Statistical package for social science
TCA	Trichloroacetic acid
USDA	United States Department of Agriculture
VSI	Viscerosomatic index
WG	Weight gain
WHO	World health organization

PENGGANTIAN SERBUK IKAN DENGAN SERBUK USUS AYAM YANG DIPROSES DAN SERBUK KACANG SOYA DALAM PEMAKANAN IKAN HARUAN, *Channa striata* (Bloch, 1793) JUVENA

ABSTRAK

Kajian ini dijalankan untuk menilai serbuk usus ayam yang diproses (P-POM) dan serbuk kacang soya (SBM) sebagai pengganti serbuk ikan (FM) bagi menggalakkan pertumbuhan dan kelangsungan hidup ikan haruan, *Channa striata* juvana. Tiga eksperimen telah dijalankan. Pertama, penggantian serbuk ikan dengan serbuk usus ayam yag diproses, kedua, penggantian serbuk ikan dengan serbuk kacang soya dan terakhir penilaian pemakanan daripada gabungan serbuk usus ayam yang diproses dan serbuk kacang soya. Dalam kajian pertama dan kedua, ikan (berat awal, 13 g) diternak dalam 30 tangki gentian kaca hitam (500l) dan diberi makan sepuluh isonitrogenous (protein 40% mentah) dan isokalorik (18.90kJ / g tenaga kasar) dalam tiga replikasi. Diet ujian adalah kawalan (tidak ada pengganti), 25%, 50%, 75% dan 100% pengganti dengan sama ada P-POM atau SBM, masing-masing. Ikan diberi makan dengan diet ujian dua kali sehari sehingga kenyang selama 12 minggu. Keputusan menunjukkan tiada perbezaan yang ketara ($P > 0.05$) dalam survival ikan tetapi prestasi pertumbuhan dan penggunaan makanan menunjukkan trend dengan peningkatan penggantian P-POM. Ikan yang diberi makanan 75PPOM jauh lebih tinggi dari segi berat badan, SGR dan PER berbanding dengan semua rawatan lain sementara FCR dalam ikan yang diberi makan 75PPOM adalah jauh lebih rendah ($P < 0.05$) berbanding dengan rawatan lain. Nilai HSI dan VSI juga didapati paling tinggi pada ikan yang diberi makan 75PPOM. Di samping itu, aktiviti enzim pencernaan protease, lipase dan amylase menunjukkan peningkatan trend dengan penggantian P-

POM dalam diet. Keputusan dari percubaan makan ini menunjukkan bahawa protein P-POM boleh menggantikan protein FM 75% dalam diet untuk juvana snakehead tanpa menjejaskan kelangsungan hidup, prestasi pertumbuhan dan penggunaan makanan. Keputusan dari diet SBM menunjukkan bahawa peningkatan SBM menunjukkan prestasi pertumbuhan yang lemah, pemanfaatan makanan, bahan kering dan pencernaan protein. Ikan yang diberi 25% penggantian SBM (25SBM) menunjukkan prestasi pertumbuhan yang paling tinggi berbanding dengan rawatan lain kecuali diet kawalan. Indeks hepatosomatik (HSI) dan indeks viscerosomatik (VSI) meningkat dengan peningkatan penggantian makanan kacang soya dalam diet. Walau bagaimanapun, aktiviti enzim pencernaan protease, lipase dan amilase menunjukkan trend menurun dengan penggantian SBM dalam diet. Dalam kajian ketiga, P-POM digunakan untuk menggantikan 25% SBM pada pelbagai peringkat dalam isonitrogenous (40% protein mentah) dan isocaloric (18.90kJ / g tenaga kasar). Ikan (berat awal, 18 g) dikultur dalam 18 tangki gentian kaca hitam dalam tiga replikasi dan diberi makan hingga 12 minggu. Diet asas mengandungi makanan ikan / protein kacang soya pada nisbah 15/25. Tahap yang diuji adalah 25/0 (FSP0), 25/5 (FSP5), 25/10 (FSP10), 25/15 (FSP15), 25/20 (FSP20) dan 25/25 (FSP25). Peningkatan P-POM dalam diet meningkatkan prestasi pertumbuhan dan penggunaan makanan ikan haruan juvena. Dalam kajian ini kombinasi terbaik didapati dalam FSP10 dan kombinasi lebih daripada dua sumber protein bukan sahaja meningkatkan prestasi pertumbuhan dan penggunaan makanan tetapi juga meningkatkan kandungan protein otot dan profil asid lemak otot, aktiviti enzim pencernaan dan pekali pencernaan yang jelas.

REPLACEMENT OF FISH MEAL WITH PROCESSED POULTRY OFFAL MEAL AND SOYBEAN MEAL IN THE DIETS OF SNAKEHEAD, *Channa striata* (Bloch, 1793) JUVENILES

ABSTRACT

This study was conducted to evaluate the feasibility of lab processed poultry offal meal (P-POM) and soybean meal (SBM) alone and a lab processed and soybean meal combination as a replacement for fish meal (FM) to promote growth and survival in snakehead, *Channa striata* juveniles. A series of three experiments were conducted, firstly, the replacement of fish meal with lab processed poultry offal meal, secondly, replacement of fish meal with soybean meal and followed by a combination between lab processed poultry offal meal and soybean meal in a fish meal based diet for snakehead juveniles. In the first and second study, fish (mean initial weight, 13 g) were reared in 30 fiberglass tanks (500*l*) and fed with ten isonitrogenous (40% crude protein) and isocaloric (18.90kJ/g gross energy) diets in triplicates. The test diets were control (no replacement), 25%, 50%, 75% and 100% replacement with either P-POM or SBM, respectively. Fish were fed with the test diets twice daily until apparent satiation for 12 weeks. Results show no significant difference ($P>0.05$) in fish survival but growth performance and feed utilization showed an increasing trend with increasing of P-POM replacement. Fish fed with 75PPOM diet was significantly higher in terms of weight gain, SGR and PER compared to all other treatments while FCR in fish fed 75% P-POM (75PPOM) replacement was significantly lower ($P<0.05$) compared to other treatments. The HSI and VSI value were also found to be the highest in fish fed with 75PPOM. In addition, the digestive enzyme activities of protease, lipase and amylase showed an increasing trend with the replacement of P-POM in the diets. The results from this feeding trial indicate that P-POM protein can

replace up to 75% FM protein in the diet for snakehead juveniles without affecting the survival, growth performance and feed utilization. The results from the SBM diets revealed that increasing levels of SBM showed poor growth performance, feed utilization, dry matter and protein digestibility. Fish fed with 25% SBM (25SBM) replacement showed highest significant growth performance compared to the other treatments except the control diet. The hepatosomatic index (HSI) and viscerosomatic index (VSI) increased with increasing soybean meal replacement in the diets. However, the digestive enzyme activities of protease, lipase and amylase show a decreasing trend with the replacement of SBM in the diets. In the third study, P-POM was used to replace 25% SBM at various levels in an isonitrogenous (40% crude protein) and isocaloric (18.90kJ/g gross energy). Fish (initial mean weight, 18 g) were reared in 18 fiberglass tanks in triplicate group and fed until satiation for 12 weeks. Basal diet contain a fish meal/soybean meal protein at a ratio of 15/25. The levels tested are 25/0 (FSP0), 25/5 (FSP5), 25/10 (FSP10), 25/15 (FSP15), 25/20 (FSP20) and 25/25 (FSP25). Increasing P-POM in the diets improved the growth performance and feed utilization of snakehead juvenile. In this study the best combination was found in FSP10 and the combination of more than two protein sources not only improved growth performance and feed utilization but also improved the muscle protein content and muscle fatty acid profile, digestive enzyme activities and apparent digestibility coefficient.

CHAPTER 1

INTRODUCTION

1.1 Aquaculture

In a world where the global population has reached 7.3 billion and is expected to grow by another 2 billion to reach 9.7 billion people by 2050, the demand for fish will increase. The world aquaculture production continues to grow each year to supplement supply from capture fisheries and according to a report by FAO (2012), the world aquaculture production reached 90.4 million tonnes, including 66.6 million tonnes of food fish and 23.8 million tonnes aquatic plants in 2012. The volume of production increased at an average rate of 8.6% per year between 1980 and 2012.

In the aquaculture industry, feed generally takes up the largest operating cost and can reach up to 50% (El Sayed, 2004). Eventually, protein makes up the biggest proportion and is the most expensive component of aquafeed and is responsible for the high production cost (Catacutan & Coloso, 1995; Lazo, Davis & Arnold, 1998). Fish meal is the main protein source in aquaculture feed especially for carnivorous fish species. However, due to high cost, fluctuating supply, unavailability and shortage of quantity (Kristofersson & Anderson, 2006), aquaculturists continuously searching for alternative protein sources as a replacement to fishmeal. The protein sources must be less expensive, good in quality and also readily available as a substitute for fish meal in the diets. There are actually several successes regarding the usage of animal protein as the replacement of fish meal in diets for warm water fish species. Animal proteins such as poultry offal meal, blood meal, feather meal as well as plant protein sources such as soybean meal are some of promising alternative protein sources (Nengas et al., 1999; Tidwell et al., 2006; Ai et al., 2006; Hussain et al., 2011).

Poultry offal meal (POM) or poultry by product meal (PBM) are waste that can be obtained from poultry industry. They are high in protein content and also contain majority of essential amino acids. They contain about 55-74% protein content, 10-19% lipid and 11-23% ash (Dong et al., 1993). In addition, the poultry offal meal is also reported to have similar nutrient profile similar to fish meal (Emre et al., 2003; Shapawi et al., 2007; Omole et al., 2008; Ismail et al., 2013). Previous studies showed that poultry offal meal can replace up to 75% or even 100% of fish meal without affecting the growth of European sea bass and gilthead sea bream (Alexis et al., 1997; Nengas et al., 1999; Takagi et al., 2000). Giri et al., (2010) conducted a study by using *Clarias batrachus* fingerling in a replacement of fishmeal with poultry offal meal in the diet and found that after 84 days of feeding, the growth for the fish fed with replacement of poultry offal meal increased compared to the fish fed the control diet. Thus, poultry offal meal can be considered as a viable protein source to replace fish meal (Webster et al., 2000; Muzinic et al., 2006; Thompson et. al., 2007).

Besides poultry offal meal, soybean meal is a common protein source used to replace fish meal for many fish species due to its high protein content, low carbohydrate and fiber, high digestibility and good amino acid profile (NRC, 1993; Gatlin et al., 2007; Hien et al., 2015). It is also less expensive and readily available (Hardy, 2006). In addition, soybean meal has low phosphorus content compared to fish meal (NRC, 1993) thus has minimal negative impact on local waterways. The successful replacement of fish meal with soybean meal has been reported for common carp, tilapia, channel catfish, salmonids, red drum, striped bass and marine shrimp (Cremer et al., 2008). FM can be replaced by SBM up to 30% in diet for snakehead (Be & Hien, 2010) and knife fish (Dan et al., 2013) and 20% in diet for spotted rose snapper, *Lutjanus guttatus* (Steindachner) (Catacutan & Pagador 2004).

Channa striata is a freshwater fish that is also known as snakehead or 'haruan' in Malay. It inhabits almost all freshwater areas from small ditches to rice fields, rivers and lakes across tropical and subtropical Asian countries from Pakistan and India to Southeast Asia and Southern China (Mohsin & Ambak, 1983; Hossain et. al., 2008). It belongs to the Channidae family which has been around from 50 million years ago with an origin from the ancient Himalayan valley (Madeleine, 2004). There are about 30 species reported worldwide from this family, and seven are found in Malaysia (Lee & Ng, 1994).

C. striata is an obligate air breather and they can survive during dry season by burrowing in the bottom mud of lakes and swamp. It is a popular freshwater carnivorous fish, commonly consumed as food fish and also known for its pharmaceutical and medicinal value in post operational recovery and wound healing for mothers after delivery (Barakbah, 2007), act as therapeutic agent which effectively cure wounds, relieving pains and also boost or stimulate energy among the sick and older people (Abdullah et al., 2010).

1.2 Problem statement

In aquaculture, dietary protein plays a very important role in growth and tissue development of fish species (Kim & Lee, 2004), and also influences fish weight gain (Sheng & He, 1994). Therefore, the amount of protein included in the diet of fish should be taken into consideration in order to promote feed efficiency and growth performance. Dietary protein is the most costly nutrient component of fish feeds (De Silva & Anderson, 1995). Fish meal is a common ingredients used globally as dietary protein in formulated fish feeds (Williams & Barlow, 1996; Hardy & Tacon, 2002; Krishnankutty, 2005; Yigit et al., 2006). However, the use of fish meal in aquafeeds is not sustainable due to high cost, uncertain availability and variation in quality (Krishnankutty, 2005; Muzinic et al., 2006; Goda et al., 2007; Subasinghe & Phillips, 2007; Tacon & Nates, 2007). In addition, overexploitation of pelagic fish for fish meal production caused a major impact on the marine ecosystem as a whole, thus resulted in the extinction of important target species.

As the world hunger crisis grows, increasing aquaculture production could be a way to ease food security problems. However, the culture of carnivorous fish species requires additional attention because of the higher demand for animal protein especially fish meal in carnivorous fish species. Therefore, there is an urgent need to identify other more cost effective protein sources to minimize or lessen the use of fish meal in fish diets. To date several animal and plant protein sources have been used in formulating the practical diets for warm water fish with varying degree of success.

Snakehead is a carnivorous fish species thus requires high level of protein in their diets. Previously, a study conducted on *Channa striata* fry showed that 55% dietary protein promote better weight gain, specific growth rate and also better muscle protein deposition (Mohanty & Samantaray, 1996). Another study revealed that 49.72% protein and 13.54% lipid promotes better growth for *Channa striata* fry (Srivastava et al., 2012). For juvenile *C. striata*, they require about 40% to 45% of protein sources in order to obtain an optimal growth. This results indicate that snakehead require high amount of protein in their diets to support better growth.

Furthermore, literature showed that protein requirement in carnivorous fish is higher compared to omnivorous and herbivorous fish species (German et al., 2004). Therefore, alternative protein sources should be identified so that the usage of fish meal can be minimized in fish diet and at the same time the feed cost can be reduced. Thus, poultry offal meal and soybean meal are some of the promising alternative protein sources to replace fish meal in the diets of snakehead juvenile. These alternative ingredients may allow for a less expensive fish feed thereby improving profitability.

1.3 Objectives

The general objective for this study is to evaluate the effects of replacement of fishmeal with lab processed poultry offal meal and soybean meal alone and the combined effect of lab processed poultry offal meal with soybean meal in the fish meal based diets on growth and feed utilization in snakehead, *Channa striata* juvenile.

Specific objectives:

1. To develop a simple farmer friendly processing method of lab processed poultry offal meal for small scale farming.
2. To determine the optimum level of replacement of fishmeal with lab processed poultry offal meal for the growth performance and feed utilization of snakehead, *Channa striata* juvenile.
3. To determine the optimum level of replacement of fish meal with soybean meal for the growth performance and feed utilization of snakehead, *Channa striata* juvenile.
4. To determine the best combination of lab processed poultry offal meal and soybean meal in the fish meal based diets for snakehead, *Channa striata* juveniles.

CHAPTER 2

LITERATURE REVIEW

2.1 Snakehead

2.1.1 Biology, Morphology and Classification

Channa striata, or snakehead, is an obligate air-breathing freshwater fish which inhabits all types of freshwater water bodies from small ditches to rice fields, rivers and lakes across tropical and subtropical Asian countries from Pakistan and India to Southeast Asia and Southern China (Mohsin & Ambak, 1983; Hossain et al., 2008). It belongs to the Channidae family which has been around from 50 million years ago with an origin apparently from the ancient Himalayan valley (Madeleine, 2004). To date, there are around 33 snakehead fish species native to Asia, and among them, eleven species are biologically and commercially important for aquaculture production and biodiversity conservation throughout the region (Rahman & Awal, 2016) and seven are found in Malaysia (Lee & Ng, 1994). In Peninsular Malaysia, three main stocks of wild *C. striata* have been described in North-West Penang, North East Kelantan and South Johor corridors (Mat Jais et al., 2009).

Channa striata has a pair of suprabranchial cavities for aerial respiration which make them very hardy and can endure for long period out of water (Hughes & Munshi, 1973; Rahman & Awal, 2016). On the top of its pharynx, the fish has also a pair of cavities, which have folded linings, richly supplied with blood vessels for taking in air. These additional respiratory organs allow these fishes to survive out of water or migrate from one place to another. They are therefore called “live fishes”. This characteristic is valuable for marketing, because live snakehead offer much higher prices compared to dead fish (Wee et al., 1982; Qin & Fast, 2003; Rahman & Awal, 2016).

Plate 2.1 Snakehead, *Channa striata* (Original source taken during study period)

Classification

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Gnathostomata
Class	Actinopterygii
Order	Perciformes
Family	Channidae
Genus	Channa
Species	*Channa striata*

Synonym: *Ophicephalus striatus*, Bloch, 1793

Ophicephalus vagus, Peters, 1869

Ophicephalus stratus var. qualamudensis, Gianferrari, 1930

Snakehead is a well-known carnivorous freshwater fish. In their natural habitat, they feed on fish, snakes, frogs, insects, earthworm, tadpoles, snails and also crustaceans (Lee & Ng, 1994; Rahman & Awal, 2016). *C. striata* catches its prey by a quick flip motion to compensate for its lack of robustness as a swimmer (Mat Jais, 2007a). That is the reason why they prefer stagnant water compared to flowing water. *C. striata* can adapt and survive in water temperature of 11 to 40°C and they can tolerate pH value of 4.25-9.40 (Lee & Ng, 1994). In addition, they can also tolerate high ammonia concentration and can survive in a condition up to 15.7 mg of unionized ammonia per litre at pH 10 (Qin & Fast, 1997).

2.1.2 *Channa striata* Aquaculture

C. striata is a common freshwater species that is a popular food source among South East Asia (Ng & Lim, 1990; Rahman & Awal, 2016) due to its firm, white and boneless tasty flesh and easy to culture making it suitable for aquaculture (Qin & Fast, 1998; Rahman & Awal, 2016). It is commonly cultured in Thailand, Indochina, Indonesia, Philippines, China, Pakistan, Cambodia and India, for either commercialisation or just for daily consumptions (Mollah et al., 2009; Rahman et al., 2012; Rahman et al., 2013; Rahman & Awal, 2016). The unique features or characteristics of this species allows it to be cultured in cages, earthen ponds, rice field-trap ponds and tanks either in monoculture or polyculture systems (Lang, 2015).

Cage culture of this species was practiced in Cambodia using bamboo or wood with sizes varying from 18-180m^3 and the average yield is between 75-150 kg/ m^3 annually (Lang, 2015). In pond culture, the species is reported to be cultured at densities of 40-80 fish/m^2 and the annual yield can sometimes reach up to 7-156 tonnes per hectare (Wee, 1982). In addition, rice field-trap pond systems is self-recruiting using *C. striata* seeds that naturally inhabits the rice field without requiring the stocking of the seeds from the hatchery (Amilhat et. al., 2009). During the wet season, rice fields become an ideal culture system for this species as it serves as a major habitat for wild fish to feed and spawn while the trap ponds are built to provide shelters during dry season prior to harvesting (Amilhat & Lorenzen, 2005).

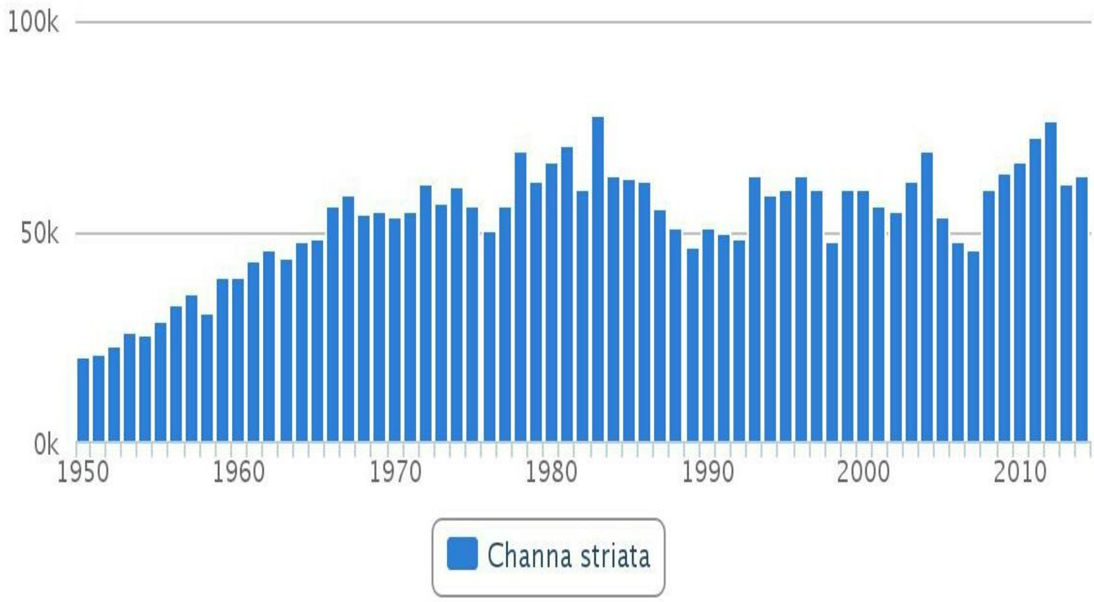

Figure 2.1 Global capture production of *Channa striata* (FAO, 2016)

Recently, overfishing, habitat loss, environmental pollution, industrial activities and urbanisation have resulted in the declined supply of *C. striata* seeds from the wild. The fluctuation of the global capture production encourage the farmers to culture this species to meet the demand (Figure 2.1). The total global aquaculture production for *C. striata* increased tremendously and by 2014 the total production of this species reached 17 847 tonnes (FAO, 2016) (Figure 2.2). Its fast growth rate, air-breathing ability, hardiness, high tolerance to adverse environmental condition characteristics and benefits as a therapeutic and pharmaceutical agent have led to the rising aquaculture of this species.

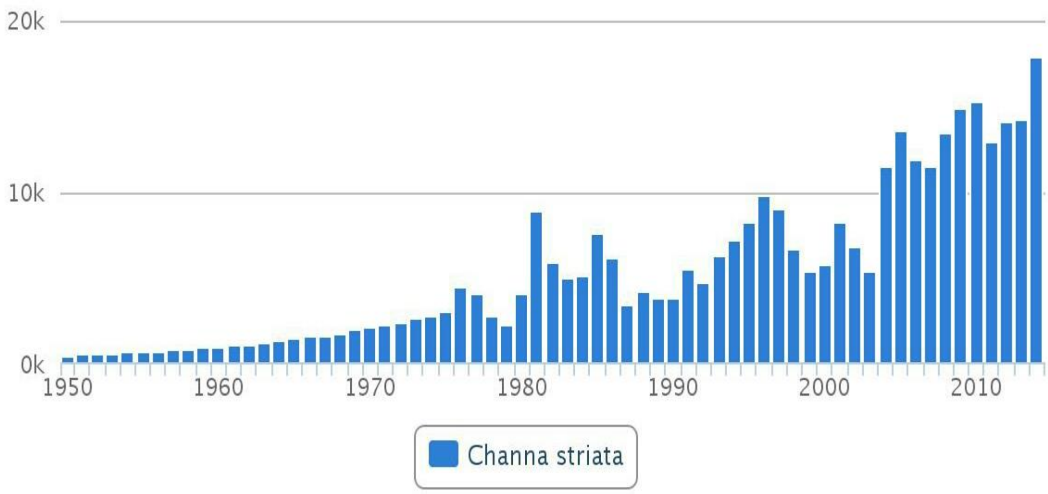

Figure 2.2 Global aquaculture production of *Channa striata* (FAO, 2016)

2.1.3 Constraints in Snakehead Aquaculture

There is little information available on the current status of aquaculture and farming practices of this species in Malaysia, as this species is not widely cultured compared to the neighbouring South East Asian countries. In Malaysia, *C. striata* is considered as a threat due to its carnivorous behaviour and hence, farmers prefer to culture other freshwater fish species compared to *C. striata* (Mat Jais, 2007a). In addition, this species faces strong competition with other freshwater species such as tilapia and catfish which are widely available and cheaper compared to *C. striata* (Mat Jais, 2007a).

It is common practice for farmers to grade *C. striata* during the culture period to reduce the cannabalistic behaviour of this fish. Captive reproduction and larval rearing of snakehead have been accomplished experimentally, but are not done on a commercial scale (Mollah et al., 2009; Rahman & Awal, 2016). Attempts have been made to develop the culture techniques for snakehead in Hawaii, USA (Qin & Fast, 1997) and successful snakehead culture will therefore require an improvement of suitable feeds and feeding approaches (Qin et al., 1997; Rahman & Awal, 2016).

In addition, the catch of *C. striata* from the natural water bodies is declining day by day and the aquaculturists are unable to go for its captive production. Consequently, seeds of this species are often collected from the natural environment making its supply unpredictable. Moreover, this practice may seriously deplete the natural stock of snakehead in the near future. Further, because of harmful effects of pesticides, chemicals and industrial wastes, natural spawning grounds are being destroyed (Mollah et al., 2009).

2.1.4 Benefits and Economic Value of Snakehead

C. striata is one of the most important source of protein in many countries especially the Asia-Pacific region such as Indonesia, Thailand, Singapore, Hong Kong, Indo-China, Taiwan, South Korea and Philippines (Wee, 1982; Mohsin & Ambak, 1983; Froese & Pauly, 2011). *C. striata* features prominently in the local diet among the Malays, the Orang Asli of Peninsular Malaysia (Haemamalar et al., 2010) and tribal communities in East Malaysia (Kodoh et al., 2009). *Channa striata* has been reported to contain majority amino acids which are higher compared to catfish, *Clarias gariepinus*, rainbow trout, *Oncorhynchus mykiss* or Atlantic Salmon, *Salmo salar* (Gram et.al., 2005). *C. striata* is also rich with arachidonic acid which serves as a precursor for prostaglandins that initiate the blood clotting process (Mat Jais et al., 1994). Furthermore, the presence of amino acid glycine which known as the key component of human collagen with the other 13 essential amino acids and Vitamin A are beneficial in the wound healing mechanism (Mat Jais, 2007a).

The popularity of *C. striata* as a therapeutic agent is related to its efficiency in treating wounds, relieving pain and boosting energy in the sick and elderly people. Besides that, mothers recuperating from normal or Caesarean delivery (Barakbah, 2007) and also patients recovering from surgical operations are recommended to eat meals containing *C. striata*. In one of the studies conducted, *C. striata* extract shows positive but mild anti- bacterial and anti-fungal response that help to speed up the healing process of wounded patients (Mat Jais, 2007a). This species also possesses a good anti-inflammatory that helps in the pain-relieving activities (Mat Jais et al., 1997; Somchit et al., 2004; Zakaria et al., 2007).

Apart from that, almost all parts of this species are beneficial for commercial purpose. Even though the mucus is being considered a valueless part of fish, it can be extracted and showed potential candidates for commercial health products (Mat Jais et al., 1997; Mat Jais et al., 1998). It also cures various skin-related problems including acne, pimples, rashes and also hormonal imbalance and skin allergy. The presence of docosahexaenoic acid (DHA) in *C. striata* is considered as an excellent nutraceutical product that can be used in repairing skin complications (Mat Jais, 2007a). Apart from that, *C. striata* is recognised to have medium level of anti- oxidant activities (Lokman, 2006) which possibly due to the presence of some major amino acid and fatty acids (Dahlan-Daud et al., 2010; Mohd Shafri & Abdul Manan, 2012). As the nutritional and pharmacological characteristics that possess by this species are well known, the demand for this species increased tremendously and in the near future there will be more requests for this freshwater fish species.

2.1.5 Nutrient Requirements of *Channa striata*

Currently it is a common practice for farmers to feed snakehead with trash fish, trash fish mixed with rice bran, vitamins and mineral (Boonyaratpalin et al., 1985). *C. striata* larvae are usually fed with Artemia and Moina since no formulated feeds are available. According to Kumar et al., (2008), the best live feed that can increase the growth and survival of *C. striata* larvae is mosquito larvae followed by bloodworm larvae and also plankton. However, the larvae can be trained to feed on the formulated feed by slowly feeding them alternately with live feed and formulated feed for certain periods of time. For example, the larvae can be fed with Artemia nauplii for the first 30 days and then changed to formulated feeds for the rest of their larval stage (Qin & Fast, 1997).

Artificial or formulated feed should contain the right lipid/protein ratio in order to ensure good growth and survival rates of *Channa striata*. *C. striata* fry needs 550 g kg^{-1} protein in their diets (Mohanty & Samantaray, 1996). However, Aliyu Paiko et al., (2010a) reported that lipid/protein ratio of 65/450 g kg^{-1} is enough for the growth and survival of *C. striata* fry. Fingerlings of *C. striata* need 130/450 g kg^{-1} lipid/ protein ratio. Another study revealed that the supplementation of diet with 49.72% protein and 13.54% lipid promotes better growth in *C. striata* fry (Srivasta et al., 2012).

2.2 Protein Sources

Dietary protein plays a very important role in growth and tissue development of fish species (Kim & Lee, 2004). However, protein is the most expensive nutrient in fish feed (De Silva & Anderson, 1995; Alarcon et. al., 2001) and to minimize feed cost, alternative protein sources should be used instead of fish meal. The partial or complete replacement of fish meal with other alternative protein sources may be the solution to this problem.

2.2.1 Fish Meal

Fish meal is widely used as the main protein source in aquafeeds especially for carnivorous fish species. Fish meal industry is one of the major industries that exist for aquaculture sector. Fish meal supply is presently stable at 6.0 to 6.5 million tons annually but as the time passed by the supply of this protein sources will decline (Miles & Chapman, 2005). Peru is one of the major producers of fish meal supply which make up almost one-third of the total world fish meal supply. Apart from that, Chile, China, Thailand, U.S.A, Iceland, Norway, Denmark and Japan are also producing fish meal for the supply of aquaculture industry. Usually, anchovies, herrings, menhaden, sardines, shads, smelt, jacks, hakes, sand lances, tunas, mackerels and cutlass fishes are being processed to produce fish meal.

High quality fish meal contains between 60% to 72% crude protein respectively. Thus, fish meal is preferred as the most suitable animal protein sources in fish diets. In addition, good amino acids profile of fish meal allows it to become the main ingredient for aquafeed. The presence of omega-3 fatty acid in fish meal such as linolenic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are necessary for larval development, fish growth and also reproduction process. Essential fatty acids are also important for the development of the skin, nervous system and brain. PUFA also assist and defence the immune system against disease and at the same time reduce the stress response in fish. Hence, because of its high nutrient content, high digestibility and palatability, fish meal is used as the main ingredient in aquaculture feed.

2.2.2 Plant Proteins

Plant proteins are the most likely candidates that can substitute fish meal in aquafeeds due to their abundance and low cost (Ayadi et al., 2012; Tankitti, 2014). Many studies have been done to investigate the suitability of plant protein as substitutes to fish meal to reduce costs; especially carnivorous fish species which rely on animal protein and have high protein requirements. Soybean meal, cottonseed meal, canola meal and lupin meal are among the plant protein sources that are widely studied to substitute fish meal in aquafeeds.

2.2.2 (a) Soybean Meal (SBM)

Soybean meal is produced by eliminating oil from the entire soybeans, toasting the flakes then grinding into meal (AgMRC, 2010; Ayadi et al., 2012). It is one of the most promising protein sources due to its high protein content (46.9% – 51.2%), crude fat (1.5% - 4.7%), crude fibre (7.1% - 8.4%) and ash (6.1% - 7.4%), respectively (Abimorad et al., 2008; Liu et al., 2009). In addition, SBM contains a fairly balanced amino acid profile (Zhou et al., 2005; Gatlin et al., 2007) but is deficient in certain amino acids such as methionine (0.50% - 0.9%) and lysine (2.8% - 4.0%) (Alam et al., 2005; Zhou et al., 2005; Gatlin et al., 2007). SBM also has significantly less phosphorous (6.5 g kg^{-1}) compared to fish meal (17-42 g kg^{-1}) (NRC, 1993; Cheng et al., 2003).

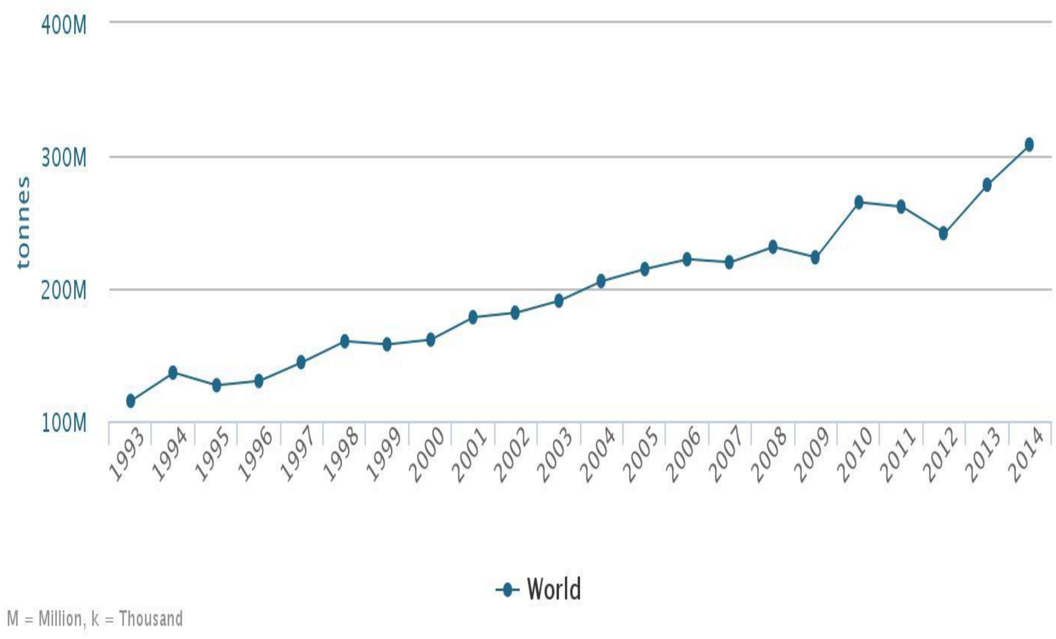

Figure 2.3 Total world soybean production (Food and Agriculture Organisation of the United Nations Statistic Division, 2015)

The total world production of soybean increased consistently and by year 2014, the total production reached 306 million metric tonnes (Figure 2.3). China recorded the highest number of production accounting for 29% of the total world soybean production followed by United States (19%), Argentina (16%), Brazil (14%), India (2%) and others (20%) (FAO, 2015). By end of year 2016, the global production of soybean was estimated to reach 338 million metric tonnes (USDA, 2016) as the demand for this plant protein increased. The price of soybean also varies with each producer country, ranging from 308.27- 869.72 USD/tonne (FAO, 2015) (Figure 2.4).

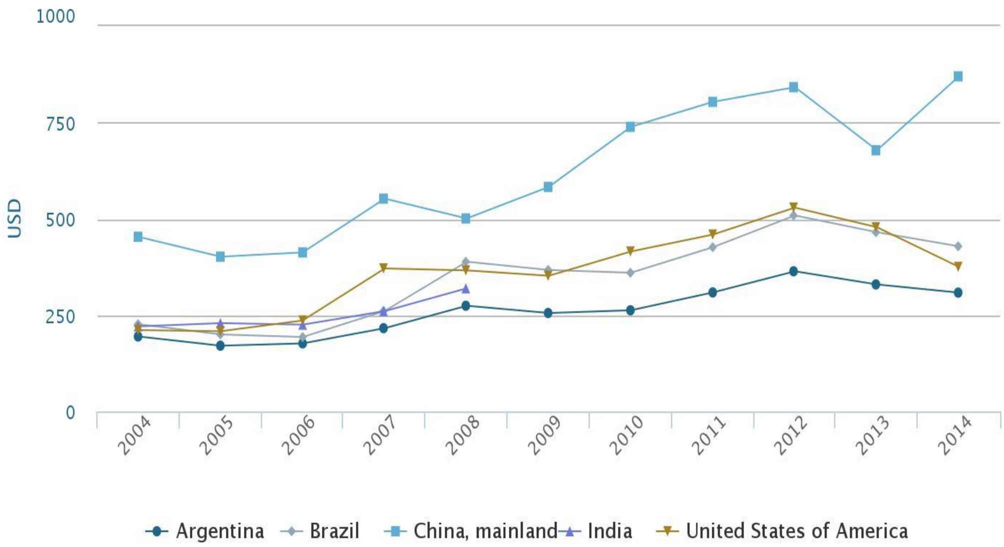

Figure 2.4 Prices of top 5 soybean producers (USD/tonne) (Food and Agriculture Organisation of the United Nations Statistic Division, 2015)

A number of studies have been done to replace fish meal with SBM either in marine or freshwater species with varying results. Red snapper, *Lutjanus argentimaculatus* growth rate, feed efficiency and mortality were not significantly affected when SBM was included at 12% up to 48% (Catacutan & Pagador, 2004) However, fish fed diets with high replacement level of 36% and 48% had lipid deposited in the liver thus declining the fish health as the haematocrit levels drop significantly. It can thus be concluded that 24% SBM could be successfully incorporated in the diets of red snapper. In addition, fish meal was successfully replaced with soybean meal at 20% and 30% replacement levels and the fish were able to feed and grow well in Mediterranean yellowtail, *Seriola dumerlii* (Tomas, 2005). Day (2000) reported that turbot, *Scophthalmus maximus*, showed no significant differences in growth or fish health when 50% of fish meal was replaced with soybean concentrate. In another study, using cobia, *Rachycentron canadum*, fish meal can be replaced with SBM up to 40% but only 18.9% was recommended as the highest possible replacement level due to poor feed conversion ratio, high lipid deposition in the liver and blood cell composition (Zhou et al., 2005).

Generally, studies on freshwater species have shown higher maximum replacement of fish meal with SBM, but studies have been focused on the omnivorous catfish and tilapia. Khan et al (2003) were able to successfully replace 100% of the fish meal with SBM in the diets of Rohu, *Labeo rohita*. In addition, Kasper et al (2007) concluded that up to 47.6% of the fish meal could be replaced with SBM in the diets of yellow perch (*Perca flavescens*) without affecting the growth performance and feed utilization of the fish. 20% of fish meal can be replaced by SBM in the diets of knife fish (Dan et al., 2013). A study conducted by Be & Hien (2010) showed that fish meal can be replaced by SBM up to 30% in the diet for snakehead.

2.2.2 (b) Another Plant Protein Sources

Cottonseed meal is a by-product obtained from the solvent extraction of oil from partially dehulled cottonseed (Sauvant, 2004). Cottonseed meal contain considerable nutrient which are crude protein 33.4 - 44.7%, crude fat 1.6 - 4.5%, crude fibre 13.0 - 28.6%, crude ash 5.7 - 7.8%. Likewise to the other plant protein sources, CSM also being reported to have low amino acids lysine and methionine (Robinson & Li, 2008) as well as high concentration of fibre (Cheng & Hardy, 2002). Cottonseed meal as the protein source for fish feed can be included up to 30% if supplemented with lysine and can replace soybean up to 60% in the diet of catfish without giving any adverse effect on the growth performance and feed utilization (Robinson & Li, 2008). In addition, CSM can be included up to 10% substitution level when 60% of the protein is contributed by fish meal in rainbow trout without giving any significant differences in growth performance (Cheng & Hardy, 2002).

Shafaeipour et al (2008) reported that Iranian canola meal could be incorporated in the diets for rainbow trout up to 30% without any adverse effect on the growth performance, feed utilization, physiological and biochemical parameter. In addition, canola meal can also be included in a diet of red seabream up to 60% without any deleterious effect on the growth performance and feed utilization (Glencross et al., 2004b). However, recent studies with Japanese seabass (Cheng et al., 2010) and cobia (Luo et al., 2012) showed that the replacement of canola meal are limited to 10% and 12.5% respectively and higher replacement levels resulted in the reduced growth performance and feed utilization.

Lupin is a protein-rich legumes which contain around 34.1 - 48.2% crude protein, 5.5 - 6.2% crude fat and a high crude fibre which is 11.6 - 19% respectively. The ash content is 2.8 - 4.9% which is quite low. The lysine and methionine content in the lupin are relatively low which are 1.4 - 2.1% and 0.2 - 0.4% respectively. Smith (2002) reported that lupin meal can replace fish meal up to 40% in the diets of *Peneus monodon* and weight gain was equal to the fish fed the diet using full fat SBM as the primary protein source. In another study by Farhangi & Carter (2001), 40% replacement of *Lupinus angustifolius* in the diets of juvenile rainbow trout showed that fish meal could be substituted with lupin meal without affecting the growth performance and feed utilization. A similar experiment was conducted with different replacement level of lupin meal in rainbow trout and it was found that the growth was significantly reduced at 50% replacement level (Glencross et al., 2004b).

2.2.3 Animal Protein Sources

Animal proteins are another alternative protein sources that can be used in aquafeeds due to their abundance, low cost, higher protein content and superior indispensable amino acid (Mohanta et al, 2013). Poultry offal meal or poultry by product meal, meat and bone meal, blood meal and feather meal are some of animal protein that can be used as alternative protein sources to substitute fish meal.

2.2.3 (a) Poultry Offal Meal (POM)

Generally, poultry offal meal (POM) is waste that can be obtained from the poultry industry. The nutrient content of poultry by product meal or poultry offal meal can vary depending on the source, quality and processing technique (Watson, 2006). High quality poultry offal meal has similar nutrient profile, amino acid and fatty acid content and high palatability properties to fish meal and they are widely used in aquaculture industry (Meeker et al., 2006). They contain about 55% - 74% protein content, 10% - 19% lipid and 11% - 23% ash (Dong et. al., 1993). Usually, poultry by product meal and poultry offal meal are golden to brown in colour with fresh poultry odour (Hertrampf et al., 2000).

Table 2.1 Nutrient composition of poultry by product meal and poultry offal meal and feather meal (Usman et al., 2006; Cotanch et al., 2006; Johnson et al., 1998)

	Ingredients		
Nutrient Composition, %	Poultry by Product Meal(PBM)	Poultry Offal Meal (POM)	Feather Meal (FM)
Protein	68.75	65.77	87.8
Lipid	19.79	17.54	10
Ash	7.5	5.87	1.9
Amino acid Composition,%			
Arginine	4.8	4.71	5.28
Histidine	1.48	0.83	1.04
Isoleucine	2.85	2.26	3.67
Leucine	5.35	4.44	6.53
Lysine	4.17	3.24	2.14
Methionine	1.45	ND	0.55
Phenylalanine	3.00	2.04	3.87
Threonine	3.09	2.62	3.64
Valine	3.64	2.75	5.67

The world poultry meat production expanded by 1.3% or 400 000 metric tonne from 2014 to 2015 mostly driven by the demand for poultry and its processing products. In 2014 alone, the total production of poultry meat reached 109 970 thousand metric tonnes and it is expected to rise in the near future (FAO, 2015) (Figure 2.5). This growth has been accompanied by the waste by product from the processing of poultry industry which causes a lot of problems to the environment. Improper disposal of poultry by product and carcasses contribute to the water quality problem especially to the area surrounding the processing plants and also in areas prone to flooding or where there is a shallow water bodies (Gerber et al., 2005).

Poultry offals released to the environment are vectors for insects, pests, bacteria and viruses, which may result in water contamination and air pollution if they are not properly managed (FAO, 2011). Processing poultry by-products into aquaculture feed is a good way to reduce the environmental problems caused by poultry processing industry. These by-products or wastes can be used as an alternative protein source to substitute fish meal in the fish diets.

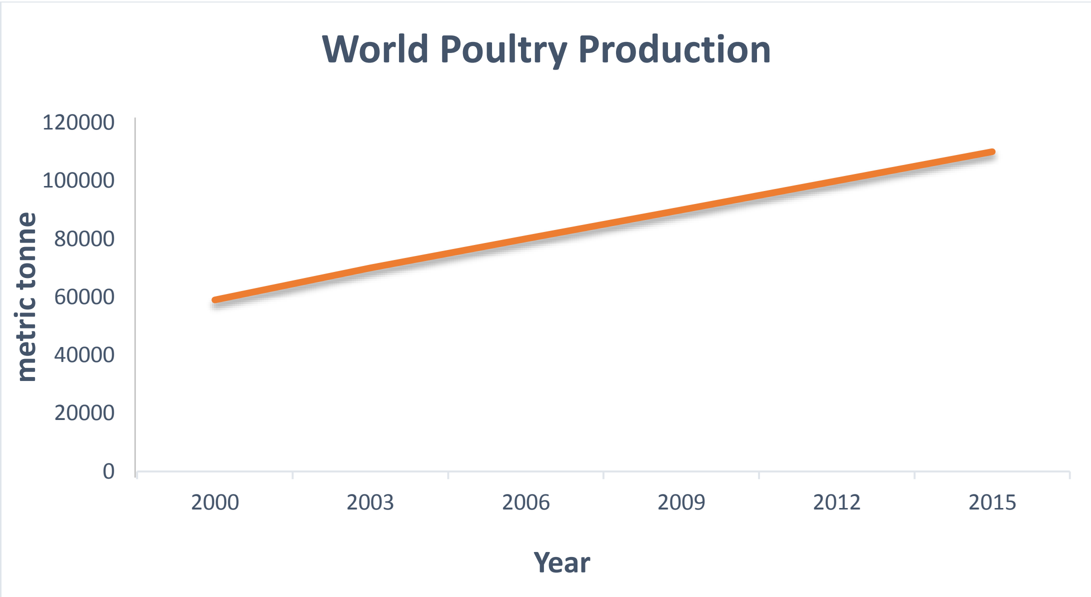

Figure 2.5 World Poultry Meat Production (OECD/ Food and Agriculture Organization of the United Nation, 2015)

Many studies have been conducted on a wide range of fish species to determine the best replacement levels of fish meal with by product from the poultry processing industry. A review of the literature (Table 2.2) shows that replacement levels differ according to fish species, feeding habit and types and quality of poultry by product meal.

Table 2.2 Summary of replacement levels of fish meal with poultry offal meal on different fish species

Species	Fish size	Type of poultry by product	% of possible replacement	References
Freshwater				
African Catfish, *Clarias gariepinus*	16 g	Chicken intestine	5	Olaniyi & Amusan,2016
	90.33-93.93g	Poultry by product meal	75	Goda et al., 2007
	2.85 g	Feather meal	20	Chor et al., 2013
Tilapia, *Oreochromis niloticus*	1.5 g	Poultry by product meal	100	Yones & Metwalli, 2015
	2.4 g	Poultry by product meal	50	Yıldırım et al., 2009
Gibel Carp, *Carrasius auratus*	4.89 g	Poultry by product meal	50	Yang et al., 2006
Mirror carp, *Cyprinus carpio*	0.39	Poultry by product meal	50	Gümüş & Aydın, 2013
	15.4	Poultry by product meal	20	Emre et al., 2003
Grass carp, *Ctenopharyngodon idella*	7.67-7.73g	Chicken intestine	100	Tabinda & Butt, 2012
Mudcatfish, *Heterobranchus longifilis*	7.26 - 94.59g	Poultry offal meal	40	Keremah, 2014